공학석사 학위논문

전기방사 멤브레인 표면에 스퍼터링을 통한 선택적 금속 코팅층을 지닌 골유도재생막의 제조

Metal coating deposition on electrospun fibers for enhanced biological performance

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재료공학부

조경일

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이 논문을 공학석사 학위논문으로 제출함

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Abstract

Metal coating deposition on electrospun fibers for enhanced biological performance

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A Guided bone regeneration (GBR) membrane has been extensively used in the repair and regeneration of damaged periodontal tissues. Fibrous membranes, consisting of a poly(lactic acid) (PLA), is a good candidate for GBR membranes for its biological and mechanical properties. However, due to its poor osteoconductivity and cellular affinity of PLA itself, its application for GBR has been limited. To achieve the better biological performance and desirable properties in GBR, various methods have been developed and investigated including e-beam deposition, microarc oxidation, ion implant doping, and thermal oxidation etc. Among them, deposition of bio-metal coating layer is a promising way to enhance the biological performance of polymer substrate.

In this study, we introduced DC sputtering deposition system as a platform for forming stable metal coating layer on electrospun PLA fiber surface. The metal

coating layers created by DC sputtering deposition have been studied for the various type of biological property enhancement agent such as osteo-conductivity, cellular affinity, and antimicrobial effect as GBR.

Firstly, the Ta is coated on electrospun PLA fiber surface through DC sputtering deposition to enhance biological performance. Various synthetic degradable biopolymers including PLA are being used as GBR materials due to their superior mechanical property to natural biopolymers however their inherent low cellular affinity limits their application. Hence, enhancement of biological property of synthetic polymer GBR is required. Therefore, Ta, which is very widely known as its excellent biological properties, is adapted as a coating material. Coating time (3 min to 5 min) and the target current (50, 75 mA) was varied to find optimum condition of the coating. And the coating stability was evaluated through SEM observation after tensile strain is given. The coating was very stable after the tensile strain was applied. *In vitro* cell test using MC3T3-E1 cells was performed to check biological effectiveness of Ta coating on the fiber surface. In terms of attachment and proliferation of cells, the biological activity of Ta coating layer was significant. This study demonstrated that the Ta coating layer created by DC sputtering deposition was stable and is effective for the enhancement of biological performance of PLA electrospun membrane as GBR.

In the second study, Antimicorbal property was adapted using Ag as a coating material. For GBR restoration, many studies indicate that the proliferation

of bacteria is one of the biggest obstacles because it disturbs the tissue regeneration and occur additional complications. Thus, we coat the Ag with mask to control the Ag coated area on Ta coated PLA fibrous membrane to expose both metals by conventional DC sputtering process. The antibacterial property of the specimens was evaluated with *Escherichia coli* and *Staphylococcus Aureus.* And the cytotoxicity of the different content of Ag was evaluated using MC3T3-E1 cells. The antibacterial activity was very effective and it increased with the area of Ag coating increased. Only Ag&Ta (20:80) sample did show the cytotoxicity in terms of cell viability. This study proved that the Ag ion from the coating layer created by DC sputtering deposition was very effective antibacterial agent.

Keywords: Porous scaffold, Poly(lactic acid), PLA, Degradable, Electrospinning, DC Sputtering, Metal coating, Tantalum, Silver, Antibacterial Effect.

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Chapter 1.

Introduction

(Theoretical review)

1.1 Potential of electrospun PLA as Guided bone membrane regeneration material

Guided bone regeneration (GBR) has been successfully applied therapy for bone defect reconstruction. In this technique, a barrier helps regeneration of new bone tissue through preventing the ingrowth of soft tissue into the necessary space for the bone regeneration. An invasion of soft tissues into the bone defect area should be avoided to retain the shape of the defect that is being reconstructed and encourage the bone growth. For GBR membrane, flexibility is first required property to adapt the shape of the bone defect and mechanical strength to conserve the empty space from invasion of soft tissue for bone regeneration. [1, 2]

An electrospinning technique is a good candidate for building GBR membrane because of its inherent flexibility in nanofibrous structure. These nanofiberous structure could enhance the absorption of protein, enzyme immobilization, cell proliferation, and wound healing because of their high specific surface area and high porosity.[3, 4] In addition, its high porosity and fibrous structure help it to mimic extracellular matrix easily. These properties are essential for the components of tissue engineering. Thus, electrospinning could have potential to be used in bone tissue regeneration engineering application.[5, 6]

Various kinds of biopolymers have been used as GBR membrane materials. Among synthetic biopolymers, there are two types of GBR materials were used, non-degradable polymer and degradable polymers. Expanded-polytetrafluoroethylene (e-PTFE) is the most commonly used non-degradable

biopolymer for GBR material. However, the GBR membrane, which is made of non-degradable polymer, needs secondary surgery to remove the GBR membrane after the defect is cured. Hence, need for degradable polymer GBR rises. In consequent, various kinds of biodegradable polymer GBR membrane were researched and developed. Among the biopolymers for GBR, poly(lactic acid) (PLA) is one of the most commonly used synthetic degradable polymer implant material for bone regeneration due to its great mechanical properties and biocompatibility compared to other synthetic degradable polymers. However, PLA has a limited use for GBR because of its relatively low cellular affinity, bone bioactivity, and stiffness. [7-9]

1.2. Limitation of electrospun PLA as Guided bone regeneration material

Poly(lactic acid) (PLA) is one of the most commonly used degradable synthetic polymer in biomedical field, especially bone regeneration area, due to its good biocompatibility and strong mechanical properties compared to other synthetic biopolymers. However, the use of PLA is quite limited because of its relatively low cellular affinity, bone activity, frequent inflammatory reaction, and bone anchor. [10] [11] [12]

Hence, various kinds of temps to enhance its biological performance such

as hybridization with bioactive inorganic materials, plasma treatment, blending with organic materials was done. But problems after treatment were occurred (e.g. poor mechanical properties). [13, 14]

1.3. The aim of this study

In this study, we adapted DC sputtering system to deposit uniform and thin metal coating layer. For the first part, tantalum-coating layer on the electrospun PLA fibrous membrane was adapted for enhancing biological performance of the membrane as guided bone regeneration membrane. And we confirmed its biological performance enhancement through *in vitro* cell test and comparison to bare PLA eletrospun membrane. In second part, Silver and Tantalum pattern coating layer was fabricated for antibacterial effect without decrease in cellular affinity. Those results were certificated with *in vitro* bacterial tests and cell test.

Chapter 2.

Tantalum (Ta) coating on electrospun PLA fibrous membrane for biocompatibility enhancement

2.1. Introduction

Tantalum (Ta) is a widely known bone implant material and coating material for bone implant for its excellent biocompatibility, biomedical safety, and biologic fixation with bone tissue. However, its extremely high mechanical elastic modulus and large mechanical incompatibility with bone tissue was a hurdle to become a ideal bone implant material. [15, 16]

In contrast, as mentioned above, PLA has been indicated for long time for its poor osteo-conductivity and cell affinity. To overcome this problem, some modification was required. In terms of lack of osteo-conductivity and cell affinity, thin layer Ta coating would be the best option for PLA.

Deposition of thin metallic coatings on the surface of electrospun fibrous membrane is a promising way to enhance their biocompatibility. A thin coating of metal layer or its oxides has been previously deposited by ion-implant doping, e-beam evaporation, micoarc oxidation, thermal oxidation and high power impulse magnetron sputtering. However, taking into account the structure of small diameter fibrous membrane made of biodegradable polymers, using plasma magnetron appears to be the most appropriate way for deposition of metal layer. [17-22]

In this work, we focus on the stable deposition of thin tantalum coating layer on the surface of electrospun PLA fiber by DC sputtering system. And the biological behavior changes due to coating layer was assessed by MTS assay and cell attachment test.

2.2. Materials and methods

2.2.1. Fabrication of electrospun PLA fibrous membrane

PLA solutions were prepared by dissolving in an organic solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma Aldrich, USA) for the preparation of electrospinning sols. The concentration of PLA in the solution is 10 wt%. The solution is then put in a 5 mL glass syringe having a stainless-steel needle with 19 gage 90° blunt end. The electrospinning setup consisted of a high voltage power supply and a digitally controlled syringe pump. As-electrospun nano-fibrous mats were collected on a metal collector (diameter of 20 cm) covered with aluminum foil. During electrospinning process, the distance between the tip of syringe and the surface of the drum was set at 15 cm, while the rotational speed of the metal collector was set at 100 rpm. For PLA solution, the flow rate and applied voltage were set at 2 ml/h and 15 kV. The thickness of the mat was 100-200 ㎛. The electrospun samples were dried under vacuum for 2 days at room temperature to evaporate the remaining organic solvent.

2.2.2. Deposition of Ta coating layer

Ta coating layer was formed by conventional sputtering process with direct-current magnetron sputter (Ultech Co. Ltd., Korea). The electrospun PLA mats were prepared in 10 × 10 mm^2 size. The specimens were placed in vacuum chamber and then the chamber was pumped down to 5 × 10^{-4} Pa using a rotary

pump and a diffusion oil pump. Subsequently, the samples were sputtered with a Ta target (purity 99.99%; Kojundo, Japan) under varying target current and time (50 mA, 75 mA and 3 min to 5 min) to find optimized condition, maintaining the working pressure at 2.4 Pa with high-purity (99.999%) Ar gas flow. The target power was fixed as 60 W and additional energy was not applied at all during the sputtering process.

2.2.3. Surface Characterization

The surface morphology of bare and Ta-coated electrospun PLA membrane were examined through field emission scanning electron microscopy (FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany). The atomic composition of each sample was investigated by energy dispersive spectrometry placed in FE-SEM (EDS/FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany). The cross-sectional images were observed using FE-SEM assisted by focused ion beam (FE-SEM/FIB, AURIGA, CARL ZEISS, Germany). Prior to the observation, the bare PLA mat was coated with a Pt coater.

2.2.4 Coating stability test

To check coating stability of Ta coating layer on electrospun PLA fibrous membrane, tensile strain was given to Ta coated electrospun PLA fibrous membrane using Instron in the tensile mode (Model 5565; Instron Corp., Danvers,

MA). The specimens were prepared in rectangular shape with a dimension of 0.1 mm × 20 mm × 50 mm. The experiments were performed with a constant strain rate of 1 mm/min. 2, 5, 10 % of strain was given to the specimen respectively.

2.2.5. *In vitro* biological analysis

The *in vitro* biocompatibility of the samples was evaluated with MC3T3-E1 cells (ATCC, CRL-2593; Rockville, MD). All the samples were sterilized by ethanol and UV light. All the samples were immersed in ethanol for 3 hr and UV treated for 1 hr. Two different samples including Bare and Ta-coated electrospun PLA fibrous membrane were chosen for *in vitro* cell test. The pre-incubated cells were seeded onto the samples at a density of 4×10^4 cells/ml for the cell attachment test and 2×10^4 cells/ml for cell proliferation test. For cell culturing, the alpha minimum essential medium (α-MEM; LM008-53, WELGENE Inc., Korea), supplemented with 10% fatal bovine serum (FBS; Gibco®, USA) and 1% Pen Strep, was used as the culturing medium, and the cells were incubated in a humidified incubator with 5% CO2 at 37 Celsius degree.

After 12 h of incubation, the morphological images of cell attachment on the each sample were observed by a FE-SEM (FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany). Prior to the SEM observation, the cells on the samples were fixed with 2.5% glutaldehyde for 10 min, followed by dehydration in graded ethanol (70%, 95%, and 100%). The samples were immersed in hexamethyldisilazane for 10 min, followed by drying the reagent in fume hood. Cell

proliferation was examined with 4 samples (n=4) for each by an MTS assay after 3 and 5 days of culturing. The cultured cells reacted with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(-4-sulfophenyl)-2H-tetrazolium (MTS, Promega, Madison, WI, USA) for mitochondrial reduction.

2.2.6. Statistical analysis

The data are presented as the mean ±SE of mean.

2.3 Results and discussion

2.3.1. Structural and mechanical analysis

As illustrated in figure 1 and figure 2, the mat was fabricated by electrospinning technique and after the membrane was fabricated, it is coated with Ta through DC sputtering deposition. Morphology of the Ta coated specimen and bare sample are shown in figure 3. The average diameter of the fiber in mat is 1 μm. In SEM images, there was no big structural change after sputtering deposition treatment. Well-deposited Ta layer was confirmed through figure 3 and figure 4 even though morphological change between bare sample and Ta coated sample was hardly found in figure 2. Ta element was found all over the specimen in EDS analysis. Figure 4 shows cross sectional image of the nanofiber through FIB technique. The Ta layer was evenly formed on the fiber (figure 4(a)) and the thickness of the Ta layer was confirmed as 70 nm (figure 4(b)).

To measure coating stability, SEM images were taken after mechanical strain was given. Figure 5 shows the morphological change after 2 %(figure 5(b)), 5 %(figure 5(c)), 10 %(figure 5(d)) strain were given. Even though there were some disconnections of the continuous coating layer along the fiber were observed, there was no sign for delamination of bulk Ta fragment. That is, when the fiber is elongated by mechanical force, some part of the Ta layer was not able to elongate enough.

2.3.2. *In vitro* biological analysis

For quantitative biological property evaluation, MTS assay was performed to check the cell proliferation rate (Figure 8). As shown in the Figure 8, Cells on the Ta coated samples showed even higher proliferation rate. The viability value was almost doubled in the Ta coated samples. For cell attachment, SEM and CLSM images were taken (Figure 9 and Figure 10). In both SEM and CLSM images, it is confirmed that the cells on the Ta coated samples demonstrated higher spreading rate and cell density. Therefore, these results indicated that the Ta coated sample showed better biocompatibility.

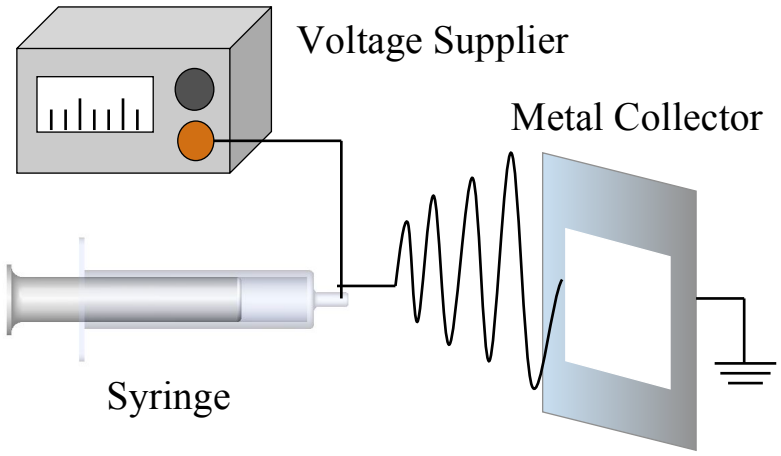

Figure 1 Schematic illustration of electrospinning process

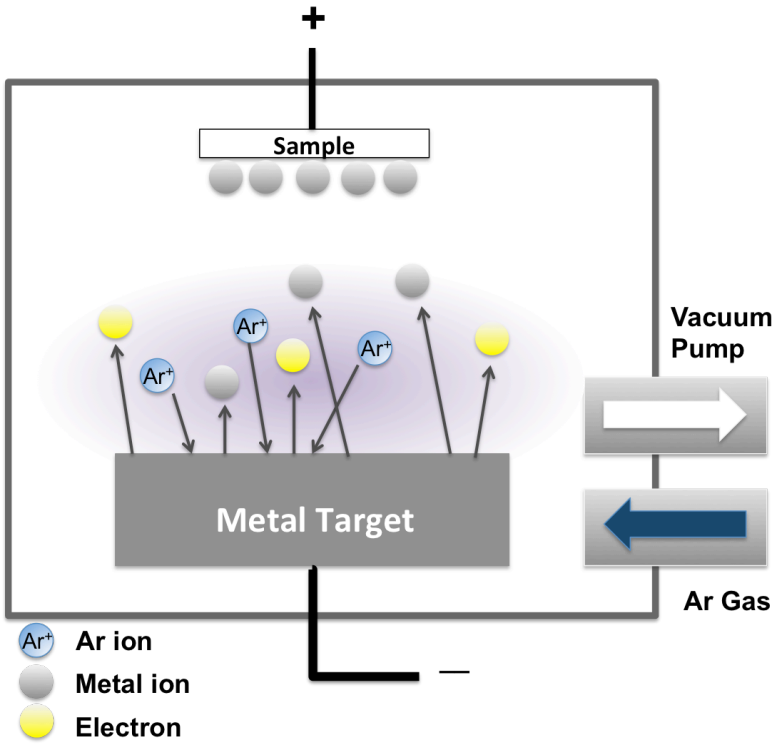

Figure 2. Schematic illustration of deposition principle for conventional sputtering process

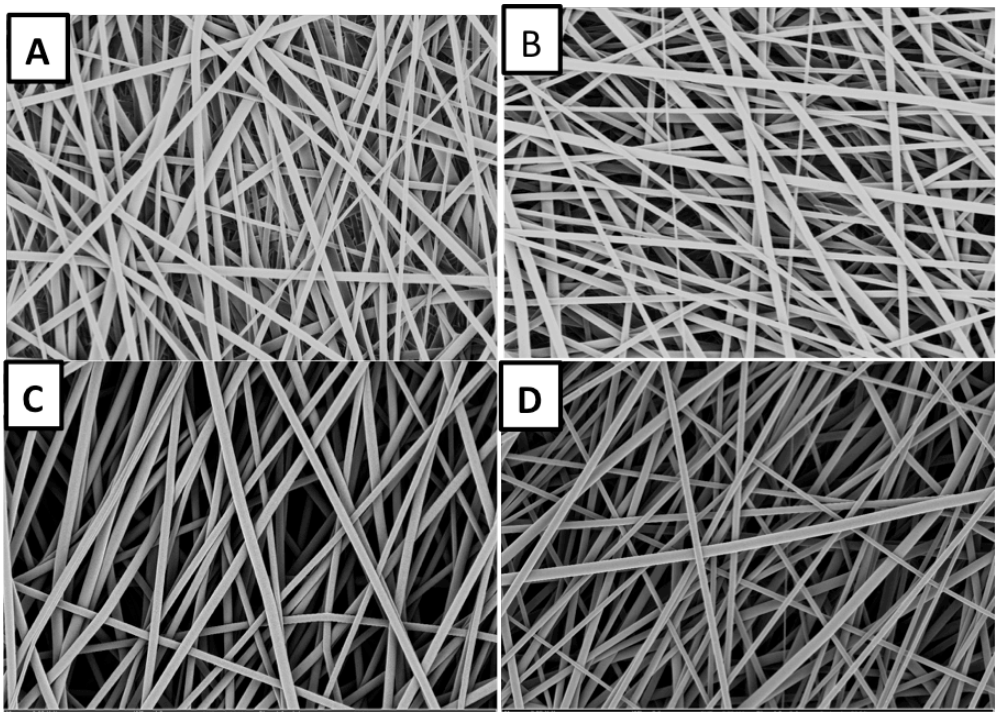

Figure 3. SEM images of A) Bare PLA membrane, Ta coated with B) 50 mA for 3 min, C) 75 mA for 3 min, and D) 75 mA for 5 min membrane

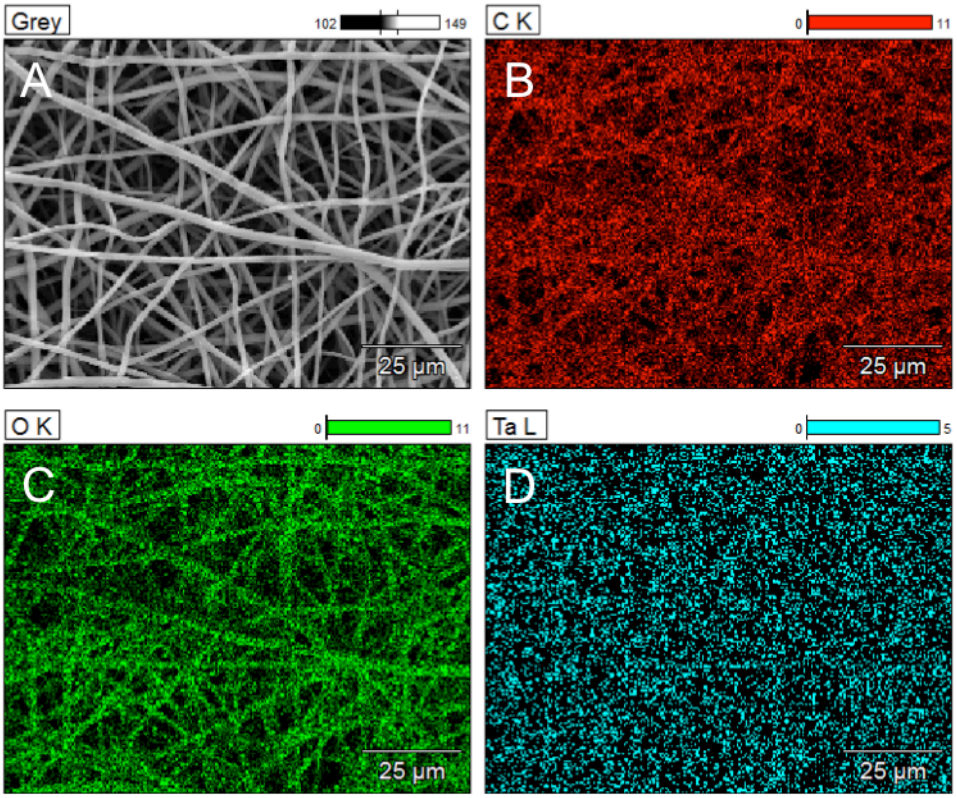

Figure 4 A) SEM image of Ta coated electrospun fibrous membrane for EDS mapping and the mapping images of B) C, C) O, and D) Ta atoms

Table 1 weight percentage and atomic percentage of each element of Ta coated electrospun fibrous membrane

Element	Weight %	Atom %
C	23.90	45.25
O	34.88	49.57
Ta	41.23	5.18
Total	100	100

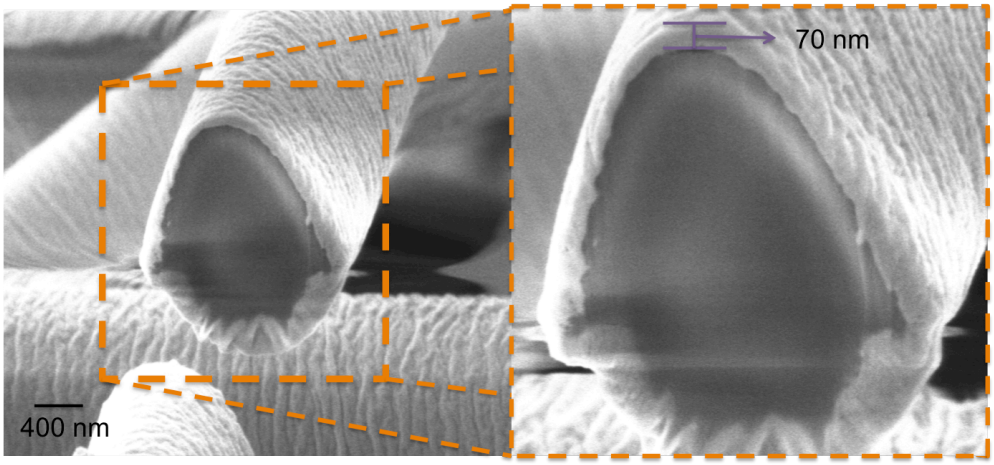

Figure 5 Typical morphology of the cross-sectional SEM image of Ta coated electrospun PLA fiber

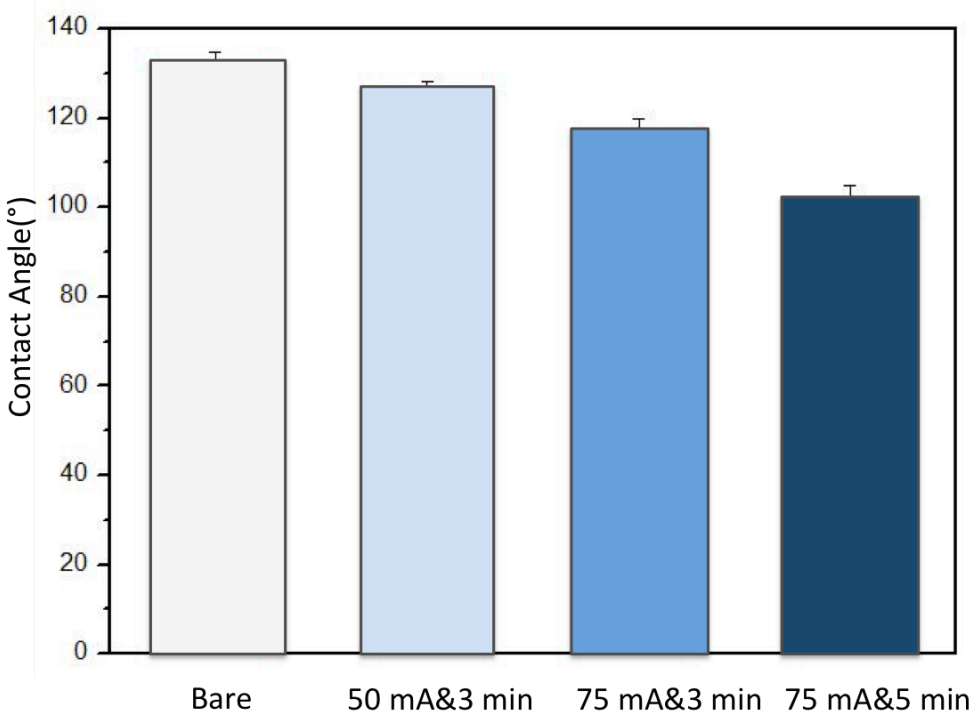

Figure 6 Contact angle of Bare PLA electrospun membrane and Ta coated membrane with various coating conditions

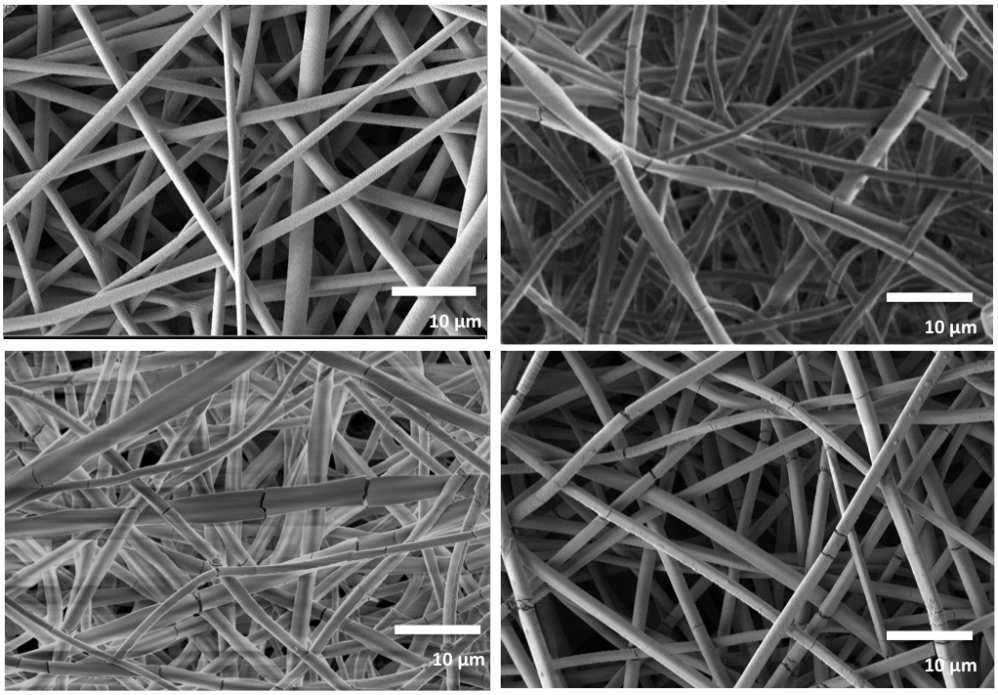

Figure 7 SEM images of Ta coated PLA membrane after applying tensile strain of A) 0 %, B) 2 %, C) 5 %, and D) 10 %

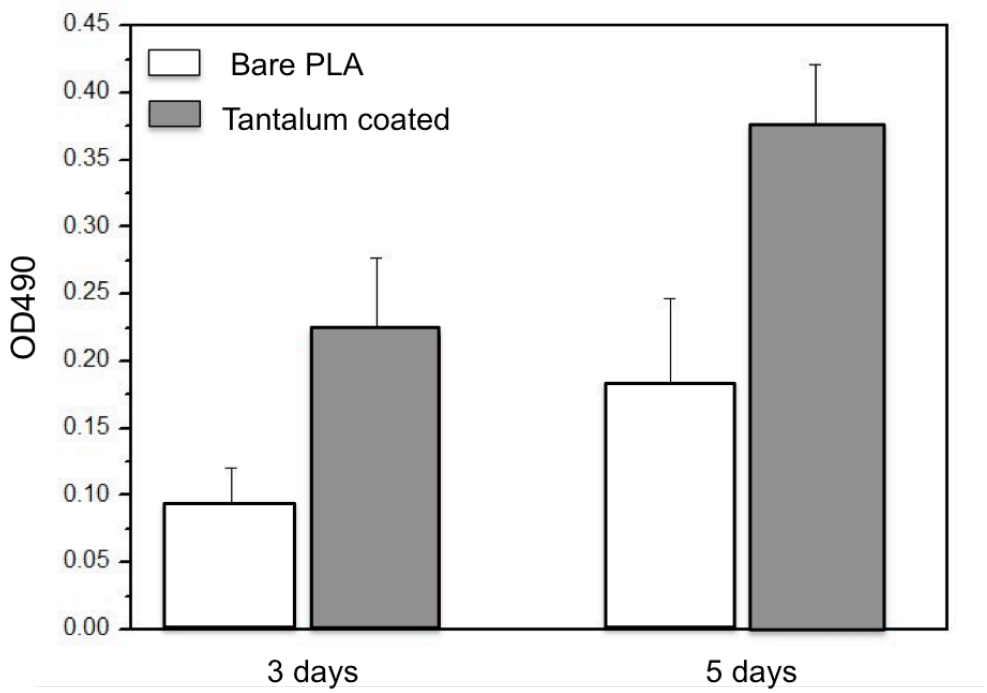

Figure 8 Cell viability measure after cultureing 3days and 5 days by MTS assay on Bare electrospun PLA fibrous membrane and Ta coated membrane

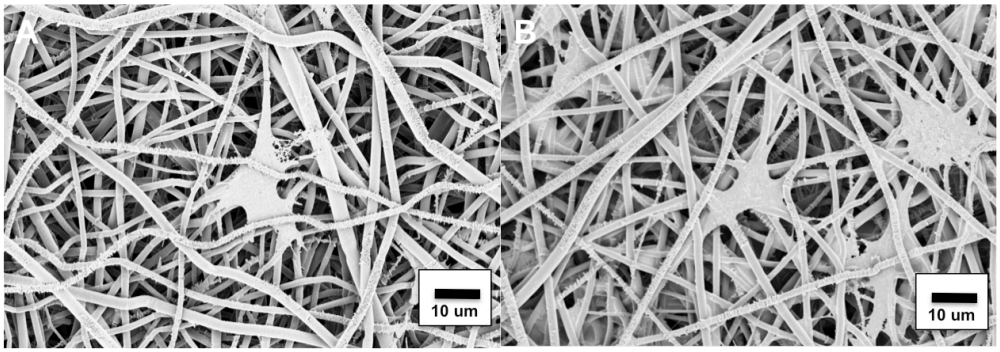

Figure 9 Typical MC3T3-E1 cell morphology on A) Bare PLA membrane and B) Ta coated membrane.

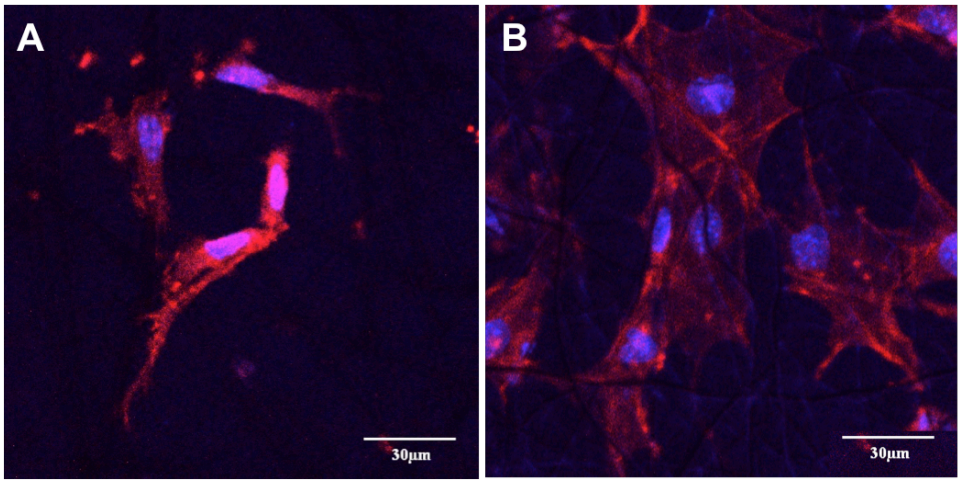

Figure 10 CLSM image of MC3T3-E1 cells on A) Bare electrospun PLA fibrous membrane B) Ta coated membrane.

Chapter 3.

Ag & Ta Pattern Coating on PLA Membrane for Antibacterial Effect

3.1. Introduction

One of the main factors that lead to unsuccessful result of bone regeneration in GBR treatment is bacterial infection such as *Porphyromonas gingivalis (P. gingivalis)* and *Actinobacillus actinomycetamcomitans*. The proliferation of bacteria on the site of bone restoration disturbs regeneration of target tissue. Thus, suppression of the proliferation of bacteria is needed to ensure successful tissue regeneration. [25, 26]

Antibiotics such as tetracycline hydrochloride and metronidazole benzoate have been used to eliminate bacteria from the site of GBR restoration. However, because of drug resistant bacteria, frequent use of antibiotics is undesirable. In terms of antibacterial effect, silver is widely known for antibacterial agent for long time. Its powerful antibacterial effect is already confirmed by many researches. Thus, we adapted the silver coating layer on the electrospun PLA fibrous membrane for fabrication of guided bone regeneration membrane with antibacterial effect.

However, Ag ion also showed negative effect to cellular activity. To overcome this problem we adapted the pattern coating using mask for two reasons. Firstly, area of silver coating is controllable. We can control the area of silver coating with the masks to find a lowest effective point for cells. Second, We can coat Ta for the rest of the specimen to compensate the negative effect for cells from Ag ion. [27-29]

3.2. Materials and methods

3.2.1. Fabrication electrospun PLA membrane

PLA solutions were prepared by dissolving in an organic solvent 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma Aldrich, USA) for the preparation of electrospinning sols. The concentration of PLA in the solution was 10 wt%. The solution is then put in a 5 mL glass syringe having a stainless-steel needle with 19 gage 90° blunt end. The electrospinning setup consisted of a high voltage power supplier and a digitally controlled syringe pump. As-electrospun fibrous mats were collected on a metal collector (diameter of 20 cm) covered with aluminum foil. During electrospinning, the distance between the tip of syringe and the surface of the drum was set at 15 cm, while the rotational speed of the metal collector was set at 200 rpm. For PLA solution, the flow rate and applied voltage were set at 2 ml/h and 15 kV. The thickness of the mat was 100-200 ㎛. The electrospun samples were dried under vacuum for 2 days at room temperature to evaporate the remaining solvent.

3.2.2. Deposition of Ag and Ta pattern coating layer

Ta coating layer was formed by conventional sputtering process with

direct-current magnetron sputter (Ultech Co. Ltd., Korea). The electrospun PLA mats were prepared in 10 × 10 mm^2 size. The prepared specimens were placed in the vacuum chamber and then the pressure of the chamber was pumped down to 5 × 10^{-4} Pa using a rotary pump and a diffusion oil pump. Subsequently, the samples were sputtered with a Ta target (purity 99.99%; Kojundo, Japan) maintaining the working pressure at 2.4 Pa with high-purity (99.999%) Ar gas flow. The target power was fixed as 60 W and no additional energy was applied.

After the deposition of Ta layer on the electrospun PLA fibrous membrane, Ag coating layer was formed by conventional sputtering process with direct-current magnetron sputter (Ultech Co. Ltd., Korea). The as-prepared Ta coated electrospun PLA mats were placed in vacuum chamber and then the chamber was pumped down to 5 × 10^{-4} Pa using a rotary pump and a diffusion oil pump. Subsequently, the samples were sputtered with Ag target (purity 99.99%; Kojundo, Japan) under 50 mA target current for 30 s maintaining the working pressure at 2.4 Pa with high-purity (99.999%) Ar gas flow. The target power was fixed as 60 W and no additional energy was applied.

3.2.3. Surface characterization

The surface of the samples was observed by using field emission scanning electron microscopy (FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany).

The atomic composition of each sample was investigated by energy dispersive spectrometry placed in FE-SEM (EDS/FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany). The cross-sectional images of the sample were observed using FE-SEM assisted by focused ion beam (FE-SEM/FIB, AURIGA, CARL ZEISS, Germany). Prior to the observation, every specimen was coated with a Pt coater.

3.2.5 Antimicrobial activity test

E. coli BL21(DE3) (E.coli; ATCC, PTA-5073, Rockville, MD, USA) strain and S. subsp. Aureus (S. aureus; ATCC 6538, KCTC 3881, Daejeon, Korea) in Luria-Bertani broth (LB broth; BD Difco™, 244620, USA) containing 50 % sterile glycerol was stored at -80 °C deep-freezer. Both strains underwent the same process for the antimicrobial test. 50 μl of the strain was seeded into 3 ml of pure LB broth medium and cultured in shaker for 12 h after seeding. After culturing, the bacteria suspension was diluted to concentration of 1.6×10^7 cells/ml of bacteria concentration with new pure LB broth medium. 1 ml of the diluted suspension was spread onto the surface of bare, Ta coated, Ag&Ta(20:80), Ag&Ta(40:60), Ag&Ta(60:40) specimen. The bacteria suspensions inoculated on each sample were incubated in 37 °C oven for 6 h with humidity control. To implement plate counting test, all the samples were washed with pure PBS two times after the incubation. After the washing the 3 ml of pure PBS in 15 ml of conical tube was prepared for detaching process and the samples were moved into the conical tube respectively.

The tubes underwent 1 min vortexing process to detach all the bacteria from surface of the samples. The bacteria in the PBS, which is detached from the samples, underwent standard serial dilution (1000 times) and spread on the LB agar plate. The LB agar plates were placed in 37 °C incubator with humidity control for 15 h to promote incubation of bacteria on the agar plates. The images of the bacteria colonies on the plates were taken by digital camera.

3.2.6 *In vitro* biological test

The *in vitro* biological performance of the samples was evaluated with MC3T3-E1 pre-osteoblast cells (ATCC, CRL-2593; Rockville, MD). All the samples were sterilized by ethanol and UV light. All the samples were immersed in ethanol for 3 hr and UV treated for 1 hr. 5 different samples including Bare, Ta-coated, Ta-and-Ag-pattern-coated (20%, 40%, 60%) membrane were chosen for the *in vitro* cell test. The cells were seeded onto the samples at a density of 4×10^4 cells/ml for the cell attachment test and 2×10^4 cells/ml for cell proliferation test. For cell culturing, the alpha minimum essential medium (α-MEM; LM008-53, WELGENE Inc., Korea), supplemented with 10% fatal bovine serum (FBS; Gibco®, USA) and 1% Pen Strep, was used as the culturing medium, and the cells were incubated in a humidified incubator with 5% CO_2 at 37 Celsius degree.

After 24 h of incubation, the morphological images of cell attachment on the each sample were observed by a FE-SEM (FE-SEM; SUPRA 55 VP, CARL ZEISS, Germany). Before the SEM observation, the cells on the samples were fixed with 2.5% glutaldehyde for 10 min, followed by dehydration in graded ethanol (70%, 95%, and 100%). The samples were immersed in hexamethyldisilazane for 10 min and the reagent was dried in fume hood. Cellular proliferation was evaluated using 3 samples (n=3) for each condition by an MTS assay after 3 and 5 days of culturing with changing culture medium every day. The cultured cells reacted with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(-4-sulfophenyl)-2H-tetrazolium (MTS, Promega, Madison, WI, USA) for mitochondrial reduction.

3.2.7. Statistical analysis

The data are presented as the mean ±SE of mean.

3.3. Results and discussion

3.3.1. Structural analysis

Figure 11 shows the typical morphology of the Ta coated and Ag and Ta

pattern coated electrospun PLA fibrous membrane. After metallic deposition, there was no sign of morphological change was detected. In Figure 12, it is confirmed that the Ag atoms were detected along the line pattern thus Well-deposited Ag layer on the fibrous structure was ensured.

3.3.2. Antibacterial analysis

The antibacterial activity of bare, Ta, Ag&Ta(20:80), Ag&Ta(40:60), Ag&Ta(80:20) coated samples is evaluated by plate counting method. Figure 13 and 15 shows the representative optic images of agar plates with the colonies of E. coli and S. aureus after 15 h of incubation in 37°C oven. A number of bacterial colonies were detected at bare and Ta coated samples and a huge number of bacteria were observed through SEM images in bare and Ta coated samples as presented in Figure 18 and Figure 19. It is confirmed that Ta coating layer, which provides favorable environment for cells, is also appropriate circumstance for bacterial growth. Meanwhile, the antibacterial effect of specimen increased as the Ag coated area increase (Figure 14 and 16).

Bare, Ta, Ag&Ta(20:80), Ag&Ta(40:60), Ag&Ta(80:20) coated samples with 12 h of E. coli and S. aureus culturing were examined using FE-SEM in order to observe the morphology of attached bacteria. Figure 18 and 19 shows the typical morphologies of E. coli and S. aureus on each surface. In the specimens that contain Ag, some dead bacteria, of which cell wall was destroyed, were detected.

3.3.2. Biological analysis

The *in vitro* biocompatibility was assessed by initial cell attachment and proliferation using a MC3T3-E1 cells to find compromising condition in terms of cytotoxicity. Bare, Ta, Ag&Ta(20:80), Ag&Ta(40:60), Ag&Ta(80:20) coated samples were evaluated by *in vitro* cell test. Figure 23 shows the representative cell adhesion morphology on each treated surface after 12 h of culturing. Unlike the bacteria, all the cells maintain their intact shape on all types of surfaces, even membrane has higher resistance to the contact with nano-scale Ag compared to bacteria membrane. In this test, the culturing condition was exactly same with bacterial FE-SEM observation test, thus, the result supports that the higher resistance of mammalian cell membrane to the contac with nano-scale Ag. Furthermore, the cell density and the level of cell spreading of Ag&Ta(20:80) samples was similar to that of bare PLA sample. [30-32]

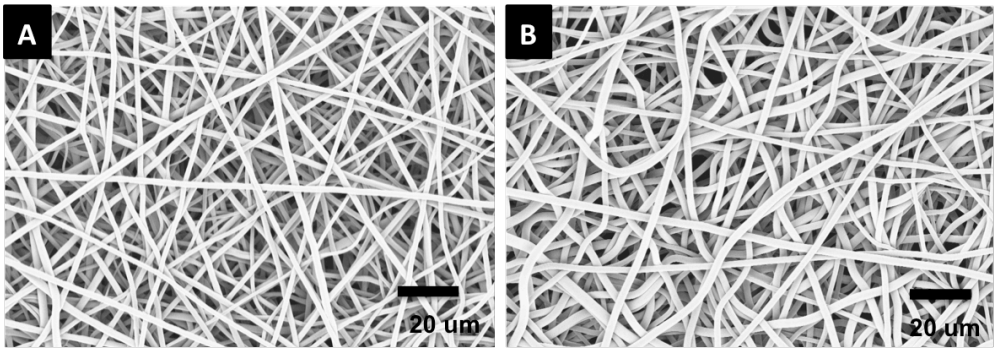

Figure 11 SEM images of A) Ta only coated PLA electrospun fibrous membrane and B) Ta and Ag pattern coated membrane

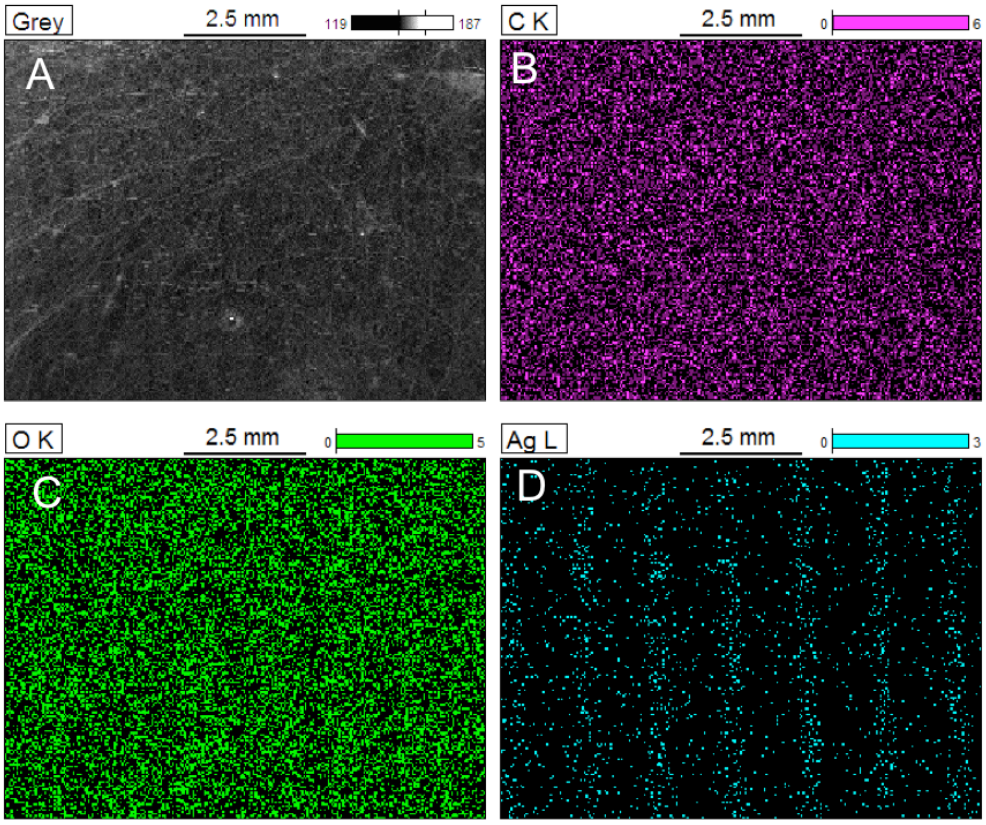

Figure 12 A) FE-SEM image of Ag pattern coated membrane for EDS mapping and the mapping images of B) C, C) O, and D) Ag atoms

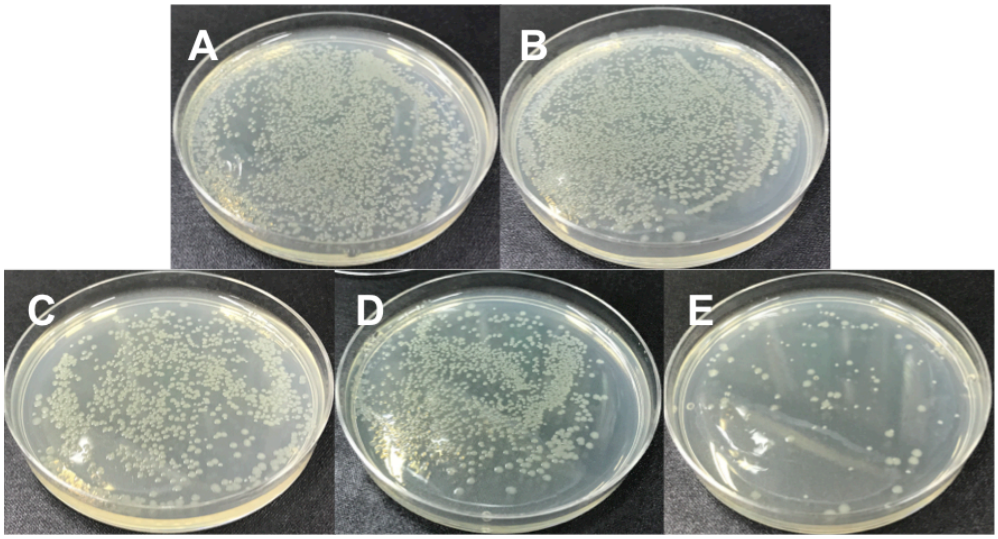

Figure 13 Bacterial colony formation of E. Coli from A) Bare B) Ta coated, C) Ag&Ta(20:80) D) Ag&Ta(40:60), and E) Ag&Ta(80:20) surface on agar plate by plate counting method after 12 h of culture on sample and 15 h of culture on agar plate.

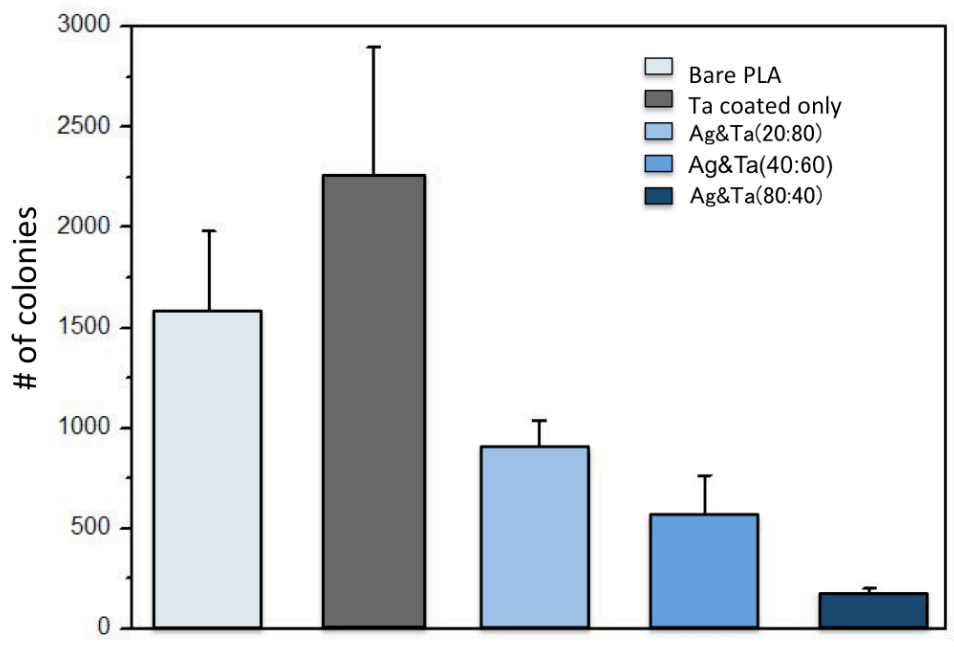

Figure 14 The number of Bacterial colony formation of E. Coli from Bare Ta coated, Ag&Ta(20:80) Ag&Ta(40:60), and Ag&Ta(80:20) surface on agar plate by plate counting method after 12 h of culture on sample and 15 h of culture on agar plate

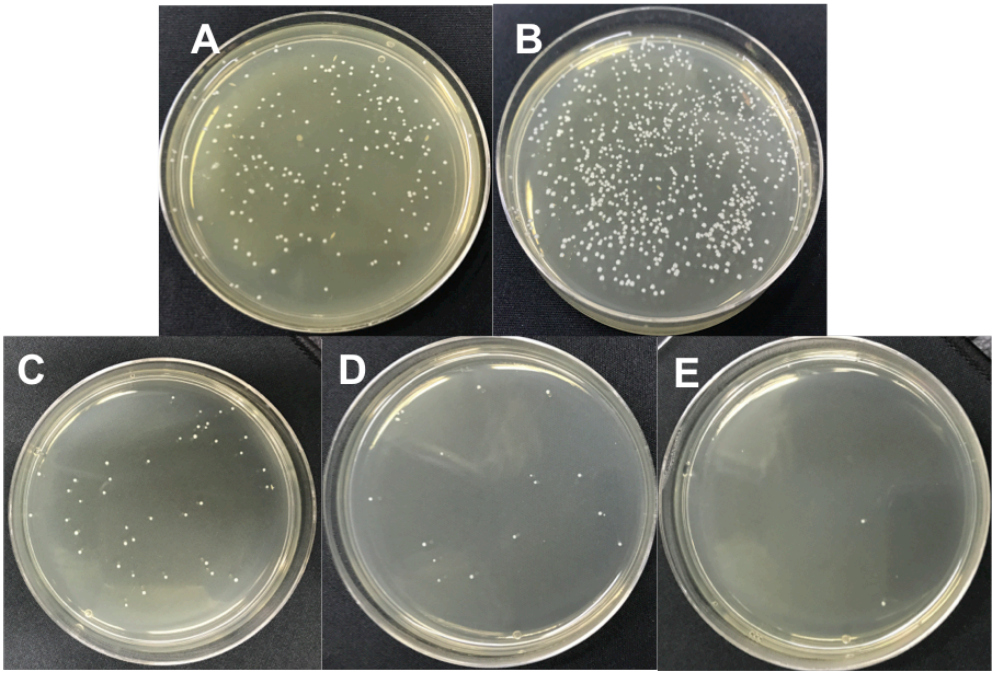

Figure 15 Bacterial colony formation of S. Aureus from A) Bare B) Ta coated, C) Ag&Ta(20:80) D) Ag&Ta(40:60), and E) Ag&Ta(80:20) surface on agar plate by plate counting method after 12 h of culture on sample and 15 h of culture on agar plate

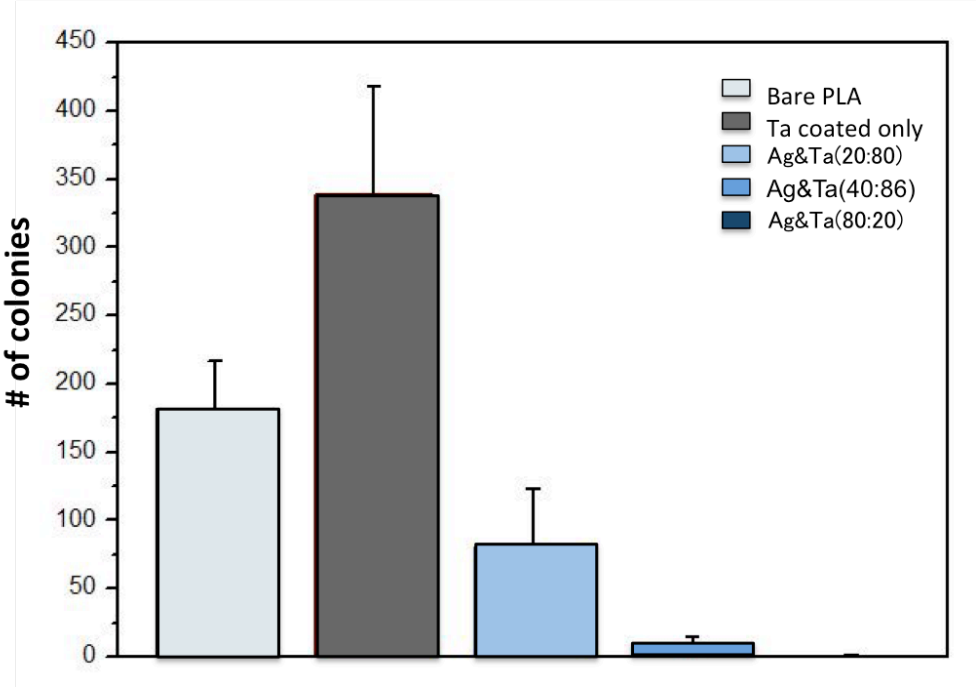

Figure 16 The number of Bacterial colony formation of S. Aureus from Bare Ta coated, Ag&Ta(20:80) Ag&Ta(40:60), and Ag&Ta(80:20) surface on agar plate by plate counting method after 12 h of culture on sample and 15 h of culture on agar plate

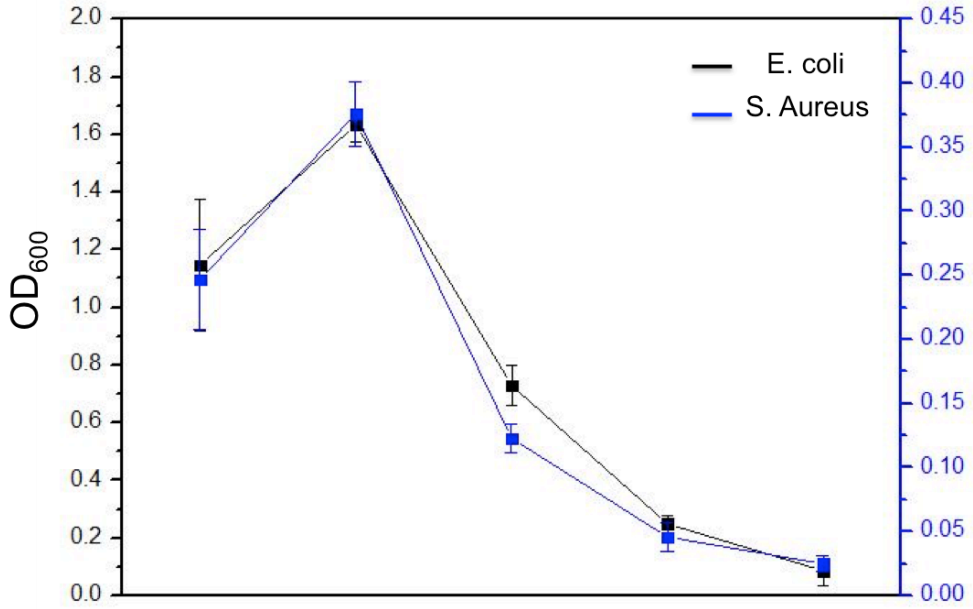

Figure 17 OD_{600} value of the medium from 6 h bacteria culture with Bare, Ta coated, Ag&Ta(20:80) Ag&Ta(40:60), and Ag&Ta(80:20)

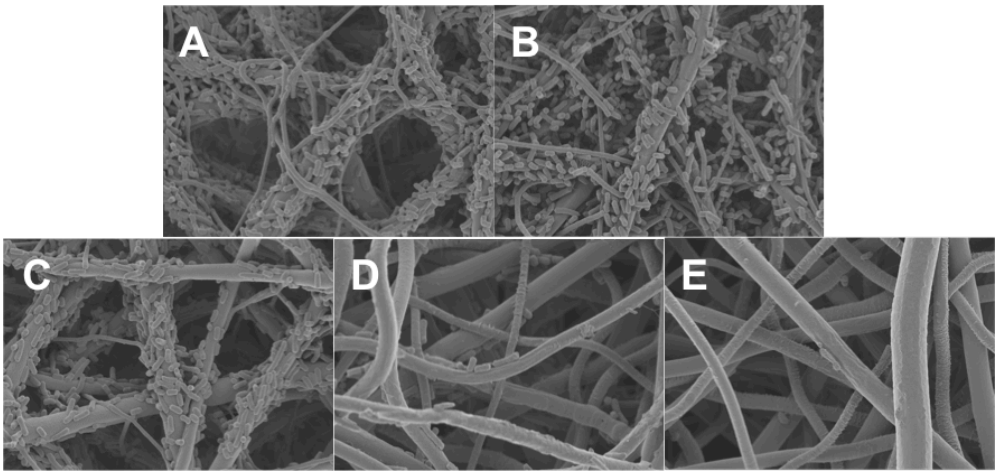

Figure 18 SEM images of A) Bare PLA, B) Ta coated, C) Ag&Ta (20:80), D)Ag&Ta (40:60), and E) Ag&Ta (80:20) after culturing E. Coli for 6 h.

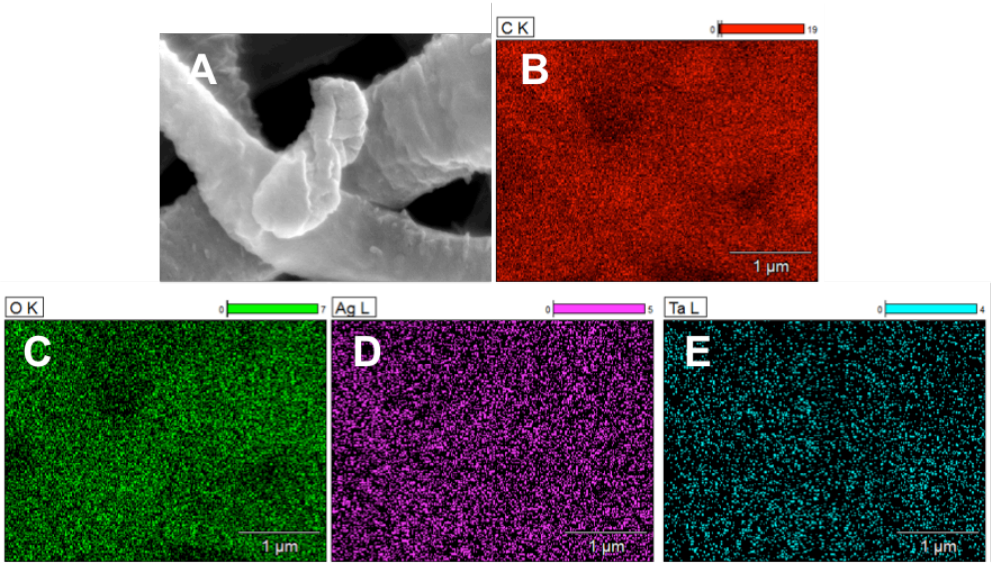

Figure 19 A) FE-SEM image of E. Coli on Ag coated fiber for EDS mapping and the mapping images of B) C, C) O, D) Ta, and E) Ag atoms

Table 2 Weight percentage and atomic percentage of each element of Ag and Ta coated electrospun fibrous membrane and E. coli.

Element	Weight %	Atom %
C	59.59	73.61
O	27.19	25.21
Ag	1.72	0.24
Ta	11.51	0.94
Total	100.00	100.00

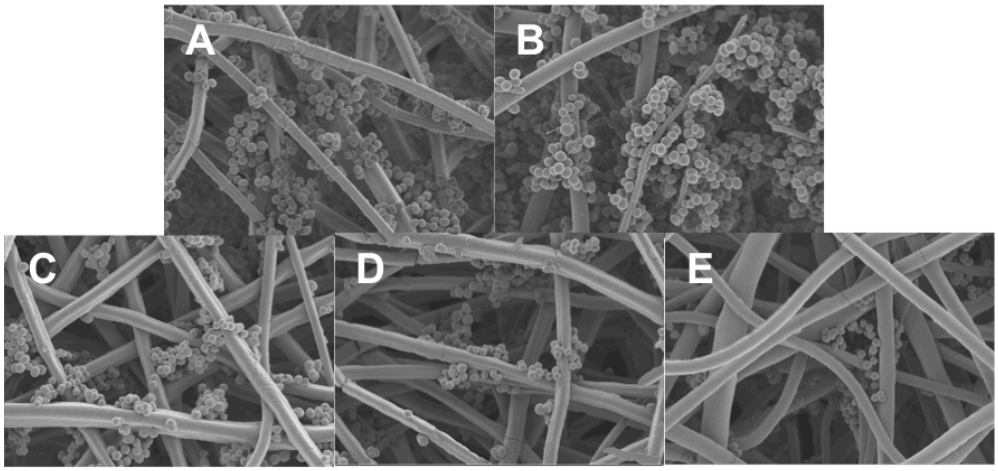

Figure 20 SEM images of A) Bare PLA, B) Ta coated, C) Ag&Ta (20:80), D)Ag&Ta (40:60), and E) Ag&Ta (80:20) after culturing S. Aureus for 6 h.

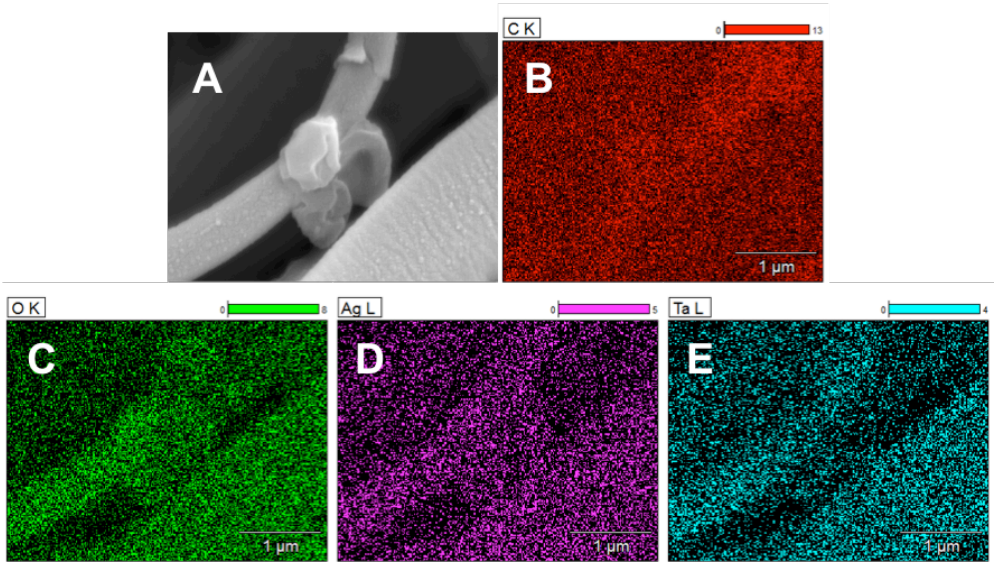

Figure 21 A) FE-SEM image of S. Aureus on Ag coated fiber for EDS mapping and the mapping images of B) C, C) O, D) Ta, and E) Ag atoms

Table 3 Weight percentage and atomic percentage of each element of Ag and Ta coated electrospun fibrous membrane and S. Aureus

Element	Weight %	Atom %
C	30.43	67.61
O	14.86	24.61
Ag	2.82	0.69
Ta	51.88	7.59
Total	100.00	100.00

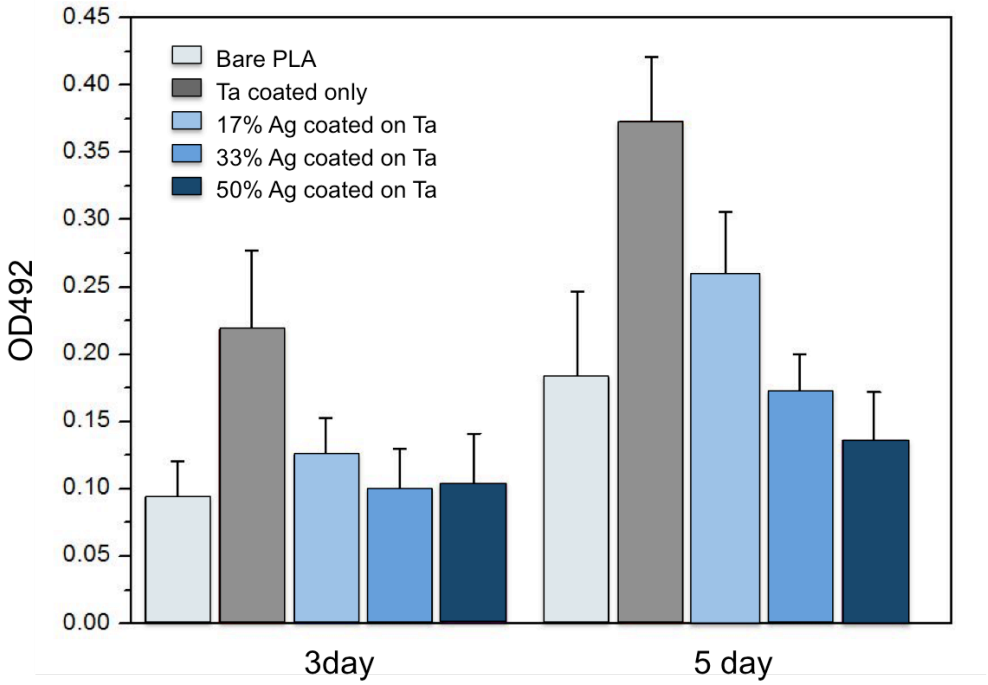

Figure 22 The MC3T3 cell viability evaluated by MTS assay on Bare, Ta coated, Ag&Ta(20:80) Ag&Ta(40:60), and Ag&Ta(80:20) after 3 and 5 days of culturing

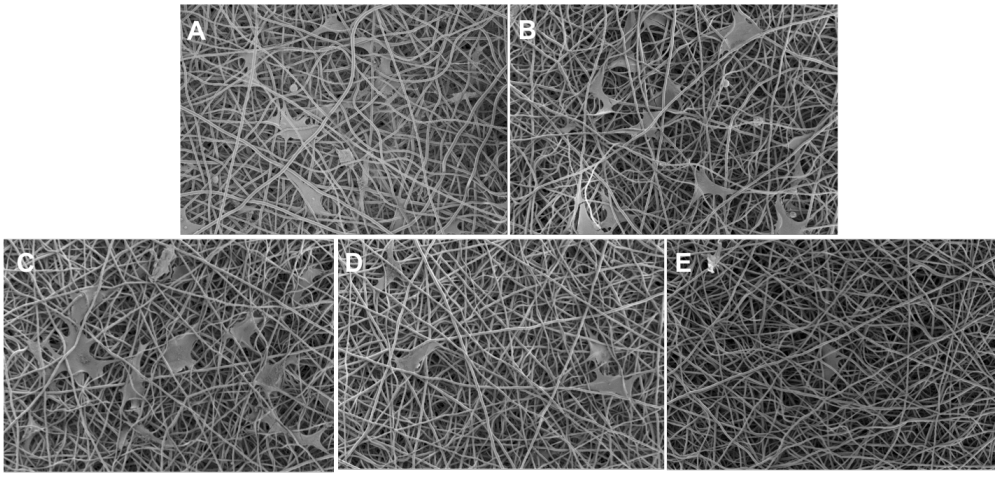

Figure 23 SEM images of MC3T3-E1 cells on A) Bare PLA, B) Ta coated, C) Ag&Ta (20:80), D) Ag&Ta (40:60), and E) Ag&Ta (80:20) respectively

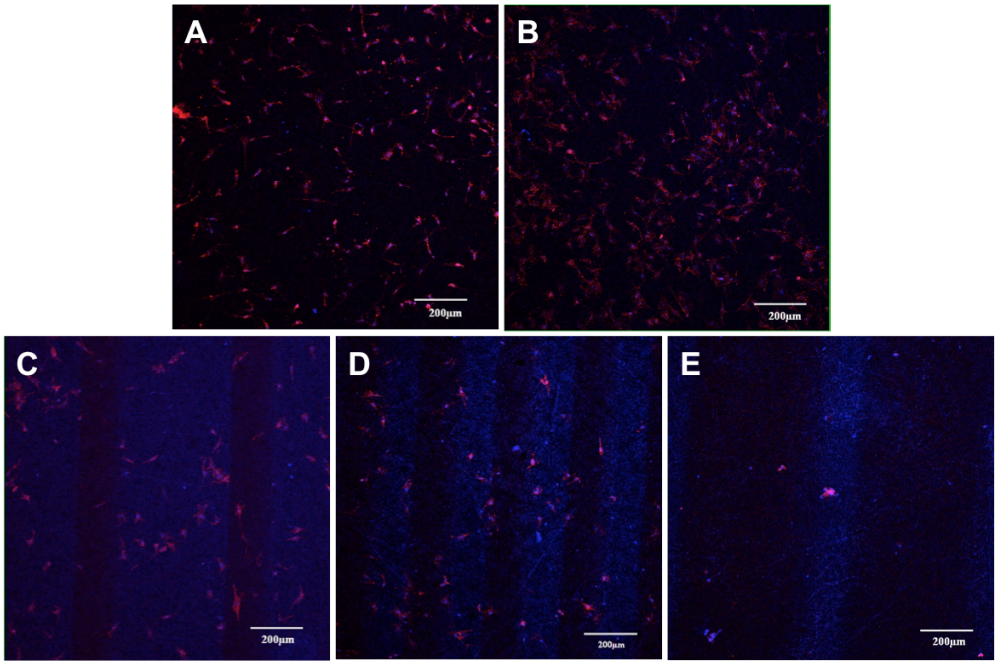

Figure 24 CLSM images of MC3T3-E1 cells on A) Bare PLA, B) Ta coated, C) Ag&Ta (20:80), D) Ag&Ta (40:60), and E) Ag&Ta (80:20) respectively

Table 4 Cell density on Bare, Ta coated, Ag&Ta(20:80) Ag&Ta(40:60), and Ag&Ta(80:20) for each part.

Specimen	Bare	Ta	Ag&Ta(20:80)		Ag&Ta(40:60)		Ag&Ta(80:20)	
			Ta	Ag	Ta	Ag	Ta	Ag
# of cells/ mm^2	68.7	131.2	70.1	35.9	61.5	25.6	15.4	5.8

Chapter 4.

Conclusion

4.1. Ta Coating on PLA Membrane for Enhancing Biological Performance.

On the PLA electrospun fiber, Ta coating was well formed consequently. The coating on the electrospun PLA fiber was uniformly deposited around the fiber. After tensile strain was applied the specimens showed no sign of delamination or fragment of coating layer even though some cracks on the Ta coating layer were detected. Thus, it is ensured that Ta coating layer, which is deposited through DC sputtering deposition, on the PLA fiber was stable. MC3T3-E1 pre-osteoblast cells demonstrated better initial attachment, higher lever of cell spreading, and higher cell density than bare PLA electrospun membrane. Similarly, in MTS assay for MC3T3 cells culturing of 3 days and 5 days, higher cell viability for both 3 days and 5days in the cells on Ta coated membrane appeared. Therefore, Ta coating layer clearly enhances the biological performance of the PLA electrospun membrane.

4.2. Ag & Ta Pattern Coating on PLA Membrane for Antibacterial Effect

In this study, to achieve additive antimicrobial activity to guided bone regeneration membrane, pattern coated Ag layer was fabricated on Ta coated surface by DC sputtering deposition system. As Ag coated area increased, the Ag content on the silver increased, that is, higher amount of Ag ion was released. The antibacterial activity test indicated that immobilized Ag on the top of the fiber is not favorable to bacteria and it might lead bacteria get killed.

The *in vitro* cell test demonstrated that the mammalian cell has higher resistance to direct contact with Ag surface and Ag ion comparison to both types of bacteria, but the Ag ion still affects to lower cell viability with regard to cellular proliferation. As the silver ion increased, the cell viability decreased after 5 days of culturing. However, Ag&Ta(20:80) showed slightly higher level of cell viability than bare PLA sample in MTS assay. Based on the results, Ag ion released from the coating layer decreased the proliferation rate of bacteria thus the PLA electrospun membrane coated with Ag could have antibacterial effect. Therefore, we can build a guided bone regeneration membrane with antibacterial effect using DC silver sputtering deposition.

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초록

골유도재생막은 손상된 골조직을 회복, 재생시키기 위하여 널리 사용되는 치료술 중 하나이다. 이 골유도재생막으로 사용되는 많은 재료 중에서 전기방사법으로 만들어진 Poly(lactic acid) (PLA) 섬유막은 높은 기계적 강도와 좋은 생체적합성이라는 장점에도 불구하고 PLA가 가지는 낮은 골전도성과 세포친화성이라는 재료적인 한계로 인하여 그 쓰임이 매우 제한되어 있었다. 때문에 이러한 재료적인 한계를 극복하고 재료의 생체 특성을 향상시키기 위하여 전자선을 이용한 증착, 미세전호산화방식을 이용한 표면처리, 금속이온의 주입, 열 산화 등의 다양한 표면처리방법들이 이용되었다. 그중에서 생체친화적인 금속층의 코팅은 고분자 기질의 생체특성을 효과적으로 증진시키는 대표적인 방법으로 알려져 있다.

따라서, 본 연구에서는 DC 스퍼터링 증착을 전기방사로 형성된 PLA 섬유구조 위에 금속 코팅층을 증착시키는 방법으로 채택하였다. 그리고 DC 스퍼터링을 이용하여 형성된 금속코팅층을 골전도성 등의 다양한 생체적합성 분석을 통하여 골유도재생막으로써의 생체특성을 평가하였다.

첫 번째 연구에서는 탄탈룸 (Ta)을 전기방사로 만들어진 PLA 섬유표면에 DC 스퍼터링을 이용하여 증착시키고, 생체적합성의 향상을 평

가하였다. 앞서 언급했듯이 다양한 생분해성 합성 고분자 중에서 PLA는 세포와의 친화도가 낮다는 것이 단점으로 지적된다. 따라서 이러한 단점들을 보완할 수 있도록 골전도성과 세포친화도가 매우 높은 것으로 널리 알려진 탄탈룸이 코팅재료로 선택되었다. 코팅의 최적 조건을 찾기 위해 코팅 시간과 타겟에 가해지는 전류를 변수로 하여 다양한 실험을 진행하였다. 그 결과75 mA의 타깃 전류에 5분간 코팅하는 것이 최적의 조건임을 발견하였다. 코팅층의 두께는 Focused Ion Beam(FIB) 방법을 사용하여 코팅의 단면을 관찰하여측정하였으며, 70 nm 두께의 탄탈룸 코팅층이 형성되었음을 확인할 수 있었다, 코팅층의 안정성은 각 시편에 인장변형을 준 뒤 주사전자현미경으로 코팅층의 표면 형상을 관찰함으로써 평가하였다. 생체적합성 면에서도 세포의 부착과 증식의 두 가지 측면에서 모두 코팅되지 않은 시편에 비해서 탄탈룸을 코팅한 시편에서 매우 향상된 것을 확인할 수 있었다.

두 번째 연구에서는 은 (Ag) 코팅층을 도입하여 항균효과를 얻는 실험을 진행하였다. 손상된 조직에서 박테리아의 존재는 그 자체만으로도 조직의 재생을 방해하는 요인으로 작용한다. 본 실험에서는 은과 탄탈룸을 동시에 노출시켜 항균효과와 생체적합성의 향상을 모두 얻기 위하여 은의 패턴 코팅을 도입하였고, 각 금속이 코팅되는 영역의 넓이를 변화시키며 골유도재생막으로서의 특성을 분석하였다. 항균실험을 대장균 (그람

음성균)과 황색포도상구균 (그람양성균)을 이용하여 진행하였다. 은이 존재하는 경우 균의 종류와 관계없이 뛰어난 항균특성을 보이는 것을 확인할 수 있었다. 뿐만 아니라 은의 함량이 증가할수록 항균효과는 증가되는 것을 확인하였다. 반면에 생체특성의 경우 은의 양이 증가할수록 감소하는 경향을 보였기 때문에 본연구에서는 은과 탄탈륨의 코팅 영역의 비율이 Ag:Ta(20:80) 일때 생체 적합성과 항균효과가 모두 우수한 최적의 조건임을 확인할 수 있었다.

본 연구를 통해서 섬유구조 본연의 구조를 변형시키지 않으면서 금속코팅층을 도입할 수 있었으며 서로다른 금속코팅층을 이용하여 필요에 맞는 다양한 생체특성을 도입할 수 있다는 것을 확인하였고, 이를 통하여 생분해성 합성 고분자가가지는 단점들을 극복하고 골유도재생막으로써의 응용가능성을 입증하였다.

Keywords: 다공성 스캐폴드, Poly(lactic acid), PLA, 생분해성, 전기방사, DC 스퍼터링, 금속코팅, 탄탈룸, 은, 항균효과.